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The 3rd International Conference for Cancer Metabolism and Therapy was successfully held at the South Hospital Conference Center of Shanghai First People's Hospital, nearly 200 international experts from the field of cancer metabolism and therapy and about two thousand local scientists attended the conference. The conference was sponsored by the Yangtze River Delta City Group Hospital Synergistic Development Strategic Alliance, the China Anti-Cancer Association Cancer Metabolism Professional Committee, the Chinese Association for Cancer Metabolism and Therapy under Chinese Medical Doctoral Association-Clinical Precision Medicine, and co-organized by the First People's Hospital Affiliated to Shanghai Jiaotong University, and Shanghai Jiao Tong University School of Basic Medicine Undertake, Translational Medicine Network, Shanghai Anti-Cancer Association Youth Council, Fudan University Affiliated Tumor Hospital, University of California, Los Angeles, Agi Hirshberg Center for Pancreatic Diseases and Hirshberg Foundation for Pancreatic Cancer Research, Dalian University of Technology, New York-Presbyterian, American Cancer Research Association (AACR). The theme of the conference was "Inheritance, Innovation, Excellence, Leading" and its aim is to create a high-end academic exchange platform to discuss new technologies, new methods, and new products in tumor metabolism, tumor immunity, tumor markers and other fields. The conference involves cancer metabolism reprogramming, metabolism and tumor microenvironment, lipid metabolism, non-metabolic function of metabolic enzymes, metabolism and epigenetics, clinical transformation, new technologies for tumor immunotherapy, clinical application of tumor immunotherapy, emerging targeted therapy, PD-1/PD-L1 technology, CAR-T technology, novel tumor protein markers, novel tumor methylation markers, ctDNA, CTC, etc. The meeting ended in a lively discussion among scientists from different levels who truly benefit from the sessions about cancer metabolism and treatment. The next meeting is planned to be held October 2 through October 6, 2019 in Los Angeles, Calif. The meeting venue will be announced accordingly in the meeting web site (www.cmt.org).

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ABSTRACTS PRESENTED

Targeting Purinergic Receptor P2Y2 Prevents the Growth of Pancreatic Ductal Adenocarcinoma by Inhibiting Cancer Cell Glycolysis

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Objectives: Extensive research has reported that the tumor microenvironment components play crucial roles in tumor progress. Thus, blocking the supports of tumor microenvironment is a promising approach to prevent cancer progression. We aimed to determine whether P2RY2 could be a potential therapy target in PDAC.

Methods: Expression of P2RY2 were determined in 264 pancreatic ductal adenocarcinoma (PDAC) patient samples, and correlated to survival. P2RY2 were inhibited in human PDAC cell lines by antagonist and shRNA, respectively, and cell viability, clonogenicity and glycolysis were determined. RNA sequencing of PDAC cell line was applied to reveal underlying molecular mechanisms. Multiple PDAC mouse models were used to assess the effect of the P2RY2 inhibition on PDAC progression.

Results: P2RY2 was upregulated and associated with poor prognosis in PDAC. Activated P2RY2 by increased extracellular ATP in tumor microenvironment promoted glycolysis and growth of PDAC. Further studies showed that the agonist-activated P2RY2 crosstalked with PDGFR mediated by Yes1, triggering PI3K/AKT-mTOR signaling that resulted in elevating expression of c-Myc and HIF1, which subsequently enhanced cancer cell glycolysis. Genetic and pharmacological inhibition of P2RY2 impaired tumor cell growth *in vivo* as well as delayed tumor progression in inflammation-driven PDAC model. Additionally, synergy was observed when AR-C 118925XX, the selective antagonist of P2RY2 receptor, and gemcitabine combined, resulting in prolonged the survival of xenografted PDAC mice.

Conclusions: These findings revealed the roles of the P2RY2 in PDAC metabolic reprogramming, suggesting that P2RY2 may be a potential metabolic therapeutic target for PDAC.

Interrelationship of Pancreatic Cancer With Diabetes, Pancreatitis and Obesity

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Pancreatic cancer (PC) is a challenging malignancy with increasing incidence and high mortality rate. It is projected that by 2030, it will be the second leading cause of US cancer mortality. Pancreatic cancer has a complex relationship with

diabetes, pancreatitis, and obesity. The mechanism is partially investigated and their contribution to pancreatic carcinogenesis are not fully understood. Diabetes can be both a risk factor and early manifestation of PC, while obesity and pancreatitis are linked to increased risk of PC. The research gaps and opportunities have been the subject of a recent National Institutes of Health (NIH) conference to guide the investigators and funding agencies. Areas reviewed include the role of altered energy metabolism in PC risk, evidence that PC can be caused by diabetes, obesity as a cause and risk factor for PC and inflammation and immune system dysfunction as a critical mechanism to PC development, progression, and therapeutic resistance. These research gaps and opportunities were summarized in the conference report (*Pancreas*. 2018:47:516–525). To address these research gaps and to foster multidisciplinary collaborations to better diagnose and characterize this interrelationship, the NIH launched the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC). The goal was to gain insight into the pathophysiology and relationship between these pancreatic diseases and to develop better methods for diagnosis, prevention, monitoring, early detection and therapy. The CPDPC will conduct studies on chronic pancreatitis in children and adults and factors that increase the risk of PC with chronic pancreatitis, T3cDM and in patients who are newly diagnosed with diabetes (*Pancreas*. 2018;47:1208–1248).

Osteoblast-Derived PKD1 Promotes the Dormancy of Prostate Cancer Cells in the Tumor Microenvironment

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Objectives: Disseminated tumor cells (DTCs) remain in the distant organs such as osteoblast niche of bone marrow without evidence of proliferation for a prolonged period of time, therefore they are resistant to chemotherapy targeting tumor cells division. Protein kinase D1 (PKD1) belongs to a family of serine/threonine protein kinases that play a key role in bone formation.

Results: Although current results demonstrated osteoblast in the tumor microenvironment promote the dormancy of tumor cells, the mechanism of which how osteoblast regulates tumor cells dormancy remains unclear. Here we show that osteoblast-derived PKD1 contributes to the prostate cancer cells dormancy in the tumor microenvironment. Overexpression of PKD1 via lentivirus infection in pre-osteoblastic cell line, MC3T3-E1 and primary osteoblast dramatically reduced the proliferation or enhanced the dormancy of co-cultured prostate cancer cells, manifested by downregulation of CDK1, PCNA, and Ki-67 in PCa cells, while upregulation of p38 phosphorylation and p21 expression. In contrast, the dormancy of prostate cancer cells was significantly reduced by osteoblast-derived PKD1 depletion during the co-culture. Interestingly, expression of dormancy related genes such as GAS6, TGF-β2, and BMP7 in osteoblast were upregulated by osteoblast-derived PKD1, while specific blockade of dormancy related proteins in osteoblast limited the ability of the osteoblasts to induce the dormancy of prostate cancer cells.

Conclusions: These findings suggest that osteoblast-derived PKD1 may play a significant role in resistance to chemotherapy through induction of prostate cancer cells dormancy in the bone marrow of tumor microenvironment.

SOCS3 Interacted With ACADVL Promotes Hepatocellular Carcinoma Metastasis

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Objectives: Suppressors of cytokine signaling 3 (SOCS3) plays crucial roles in JAK/STAT signaling pathway, signaling for negative feedback to STATs (Signal Transducer and Activator of Transcription), and is found to be inversely correlated with STAT3 expression. SOCS3 has been observed in certain human cancers, and the protein expression of SOCS3 relatively reduces in cancer tissues. However, SOCS3 expression in HCC (Hepatocellular Carcinoma) is paradox.

Methods: Cellular energy metabolism was detected by ATP assay. The mRNA expression levels were investigated by real-time PCR analysis. The expression of proteins was investigated by western blotting, immunofluorescence staining and immunohistochemical staining. We also stably knocked down the SOCS3 protein using siRNA system.

Results: Our results showed that in HCC tumor tissues, SOCS3 showed an increased expression compared with that in the adjacent non-tumor tissues, and also was high-expression in HCCLM3 cell. Then, we found that SOCS3 could interact with ACADVL, a mitochondrial intima protein, which catalyzes the first step of mitochondrial β-oxidation for long-chain fatty acids. We extracted the mitochondria and found that SOCS3 decreased in the cell with knocking down TOM70, which means SOCS3 maybe enter the mitochondria by TOM70. After knocking down SOCS3, the STAT3 and ATP did not increase a lot, respectively.

Conclusions: SOCS3 expressed in HCC tissues is different from other researches. The reason is that the hypermethylation of SOCS3 gene is found in only 30% of hepatocellular carcinoma (HCC) tissues, but the methylation-directed silencing of SOCS-3 gene promotes cell growth and migration in human HCC. SOCS3 can interact with ACADVL, but the mechanism is still not elaborate.

TNFAIP3 (A20) Inhibits Prostate Cancer Cells Migration and Invasion via Down-regulating Autophagy

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Objectives: A20, also known as tumor necrosis factor alpha induced protein 3 (TNFAIP3), is a cytoplasmic zinc finger protein that inhibits nuclear factor kappa-B (NF-kappaB) activity and prevents tumor necrosis factor (TNF)-mediated programmed cell death. Although A20 mutations are frequently found in multiple malignancies suggesting a potential role as a tumor suppressor as well, its role in cancer metastasis remains largely unknown.

Results: Here we show that A20 decreased cell migration and invasion through downregulation of autophagy in prostate cancer cells. Overexpression of A20 via lentivirus not only inhibited the migration and invasion but also downregulated autophagy in prostate cancer PC3 and DU145 cells. In contrast, knockdown of A20 by lentiviral vector enhanced the migration and invasion as well as autophagy in prostate cancer cells. Moreover, reduced tumor cell migration and invasion caused by overexpression of A20 were remarkably recovered by autophagy activator rapamycin, while enhanced tumor cells migration and invasion trigged by A20 silencing with siRNA could be antagonized by 3-Methyladenine (3-MA), a autophagy inhibitor.

Conclusions: Mechanistically, endogenous A20 interacts with autophagy related protein Beclin1, leading to reduce the expression of Beclin 1 and LC3/2. These data suggest that A20 may inhibit tumor metastasis via downregulation of autophagy in prostate cancer, and thus may provide a potential target for prostate cancer treatment.

Identification of Prognostic Biomarkers Regulated by the KEAP1-NRF2-CUL3 Axis in TCGA-Head and Neck Squamous Cell Cancer (HNSCC)

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Objectives: NRF2 is the key regulator of oxidative stress in normal cells and aberrant expression of the NRF2 pathway due to genetic alterations in the KEAP1 (Kelch-like ECH-associated protein 1)-NRF2 (nuclear factor erythroid 2 like 2)-CUL3 (cullin 3) axis leads to tumorigenesis and drug resistance in many cancers including HNSCC. The main goal of this study was to identify specific genes regulated by the KEAP1-NRF2-CUL3 axis in HNSCC patients, to assess the prognostic value of this gene signature in different cohorts, and to reveal potential biomarkers.

Methods: RNA-Seq V2 level 3 data from 279 tumor samples along with 37 adjacent normal samples from patients enrolled in the Cancer Genome Atlas (TCGA)-HNSCC study were used to identify upregulated genes using two methods (altered KEAP1-NRF2-CUL3 versus normal, and altered KEAP1-NRF2-CUL3 *versus* wild-type). We then used a new approach to identify the combined gene signature by integrating both datasets and subsequently tested this signature in 4 independent HNSCC datasets to assess its prognostic value. In addition, functional annotation using the DAVID v6.8 database and protein-protein interaction (PPI) analysis using the STRING v10 database were performed on the signature.

Results: A signature composed of a subset of 17 genes regulated by the KEAP1-NRF2-CUL3 axis was identified by overlapping both the upregulated genes of altered versus normal (251 genes) and altered versus wild-type (25

genes) datasets. We showed that increased expression was significantly associated with poor survival in 4 independent HNSCC datasets, including the TCGA-HNSCC dataset. Furthermore, GO, KEGG, and PPI analysis revealed that most of the genes in this signature are associated with drug metabolism and glutathione metabolic pathways.
Conclusions: Altogether, our study emphasizes the discovery of a gene signature regulated by the KEAP1-NRF2-CUL3axis which is strongly associated with tumorigenesis and drug resistance in HNSCC. This 17-gene signature provides potential biomarkers and therapeutic targets for HNSCC cases in which the NRF2 pathway is activated.

The Effect of IRX6 Hypermethylation in the Pathogenetic of Pancreatic Cancer

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Objectives: Pancreatic cancer is a kind of highly malignant tumor. In-depth study of the molecular mechanism and identifying novel therapeutic targets are the preconditions for pancreatic cancer treatment. The inactivation of gene expression caused by aberrant methylation of tumor suppressor gene is an important cause of promoting tumor development. Therefore, DNA methylated genes is promising to serve as a new target for the treatment of pancreatic cancer.
Methods: We predicted genes with abnormally high methylation and low expression in pancreatic cancer by bioinformatics analysis. Of 13 promising genes, we focused our studies on IRX6, a tumor suppressor gene in pancreatic cancer, for further downstream analysis, including methylation and expression. In order to further study the tumor suppressing effect of IRX6 in pancreatic cancer cells, we constructed IRX6 overexpression and knock-down cell lines through lentivirus expression vectors.
Results: We identified that IRX6 is significantly down-expressed and methylated in pancreatic tumor tissues compared to normal tissues in tissue arrays ($P < 0.05$). Through the further analysis, the low expression of IRX6 caused by the DNA methylation was proved to be negatively correlated with pancreatic tumor grade (Rs = -0.229, $P = 0.042$). Functional assays of IRX6 gene in pancreatic cancer cells indicated overexpression with IRX6 full-length gene induced significantly reduction in cell proliferation and colony formation rate, as well as the promoting effect on the cell migration, invasion and sensitivity to Gemcitabine ($P < 0.05$). Through the detection of EMT-related markers, we found that reducing the expression of IRX6 promoted the EMT process.
Conclusions: IRX6 may have tumor-suppressive effects in human pancreatic cancer and is promising to serve as a new target for the treatment of pancreatic cancer.

A Novel Detection Tool Being Used for miRNA Detection in Human Serum

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Objectives: Pancreatic cancer (PC) is one of the most malignant cancers with poor prognosis and high mortality. Therefore, the early diagnosis a crucial in the prevention and treatment of PC. Detection of PC in clinic traditionally relied on medical history, physical examination, pathological morphology, and imaging diagnosis such as CT, etc., which demonstrated less sensitive approaches be used clinically. Considering the specific expression of miRNA in PC at different stages suggested miRNAs being developed as a novel diagnostic biomarker. In this study, a novel technique of multiplex reverse transcriptase quantitative polymerase chain reaction (mRT-qPCR) was developed for detection of multiple miRNAs in a reaction in human serum.
Methods: RNA extraction from serum of subjects was performed by using phenol extraction method. Unique primers and probes were designed by using Beacon, Optimization of the mRT-qPCR was performed in various specimens.
Results: With different optimization of the probes and experimental conditions, a mRT-qPCR assay was developed successfully, which can detect simultaneously four different genes in a reaction including U6, miR159, cel-39 and a specific miRNA, including miR220, miR30, miR24a, miR23b, and miR132a. The mRT-qPCR assay was assessed based on the following parameters including accuracy, sensitivity, specificity and repeatability of detection in clinical serum samples. Currently, more than 80 clinical samples have been tested by the mRT-qPCR assay. As compared to a classical single RT-qPCR, the newly developed mRT-qPCR is superior to the signal RT-PCR in detection of miRNA in serum with an improved CV (<5%), and lower FDR (<1%), and high accuracy (100% conincidence to a reference lab).
Conclusions: The newly developed mRT-PCR is proved to be an effective way for detection of multiple genes in a reaction for analysis of miRNA in human serum.

Co-regulation of STAT3 mRNA Localization and Translation by TDP-43 and Fmrp Promotes Hepatocellular Carcinoma Metastasis

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Objectives: Transactive response DNA-binding protein 43kD (TDP-43) is a ubiquitously expressed RNA-binding protein required for early development, and it has been implicated in multiple cellular processes including cell cycle progression, apoptosis, RNA processing, alternative splicing, etc. Due to its central role in neurodegenerative disease pathogenesis, most research recently has focused on its role associated with neurodegeneration disease, however, how it affects cancer progression is still unknown.
Methods: We used RNA IP assay and FISH combined with IF to verify the relationship between TDP-43 and STAT3 mRNA. We also stably knocked down the TDP-43 protein using siRNA system, then, RNA extraction, RT-qPCR array, FISH, IF, western blotting were conduct to test the expression and localization of Stat3. Using co-immunoprecipitation, RTCA, transwell, immunohistochemical analysis and establishing a in situ liver cancer model to find the role of TDP-43 in HCCLM3 cells and human liver cancer tissue.
Results: Our results showed that TDP-43 was interacted with STAT3 mRNA and Fmrp protein in HCCLM3 cell. Meanwhile, TDP-43 can interact with many translational complexes, such as eIF4G and eIF4E. By silencing TDP-43, we saw the most of STAT3 mRNA molecules of HCCLM3 cell were located in the cytoplasm and the Stat3 expression was increased, we also found that significant inhibition of cell invasion and metastasis in HCCLM3 cells. After knocking down TDP-43, the levels of metastasis in the lungs of nude-mouse are decreased. Finally, our results have shown that the high expression level of TDP-43 was expressed in hepatocellular carcinoma tissue than para-carcinoma tissue.
Conclusions: In this study, we identified STAT3 mRNA as a new TDP-43 binding target which involved STAT3 mRNA translation through interacting with Fmrp. Knocking down TDP-43 decrease the HCCLM3 metastasis, and the TDP-43 protein level is elevated in hepatocellular carcinoma tissue, suggesting that TDP-43 may affect the hepatocellular carcinoma metastasis though interacting with Fmrp to regulate expression of Stat3.

Characterization of Hollow Hematite Sub-micron Spheres Prepared by Sol-gel

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Objectives: In this work, we report the preparation by sol-gel and the characterization of hollow sub-micron hematite spheres (α-Fe_2O_3).
Methods: For the preparation, ferric nitrate and citric acid were diluted in water and agitated to form the gel precursor without the use of templates. The oxidation was performed by annealing at different temperatures up to 600°C. The characterization was performed by X-ray diffraction (XRD) and Small angle X-ray scattering (SAXS) which revealed the phase evolution from magnetite to pure hematite. This is confirmed by thermo gravimetric analysis.
Results: Scanning electron microscope images shows the formation of the hollow spheres, with ~800 nm external diameter and ~60 nm shell thickness suggesting they are promising for encapsulation applications. The Morin transition, which is a typical property on the magnetism of hematite, is studied after annealing the sample at different temperatures. At low annealing temperatures (from 200 to 450°C) the Morin transition is affected by the magnetic domains of the secondary phases. However after annealing the sample at 600°C a clear Morin transition is observed indicating pure hematite.
Conclusions: The change of strong ferromagnetism to weak ferromagnetism is also studied from the respective hysteresis loops.

Lappaconitine Sulfate Suppresses Proliferation of Human Cervical Cancer Cells by Regulating the PI3K/Akt Signaling Pathway

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Objectives: Lappaconitine (LA), an important diterpenoid alkaloid, can be isolated from many plants of aconitum species such as *Aconitum sinomontanum Nakai*. Since lappaconitine was extracted, it has exhibited a wide range of biological activities. Lappaconitine hydrobromide (LH) is a major drug widely used in clinical for treatment of neuropathic pain as a non-addictive drug in China and as an antiarrhythmic in Uzbekistan. Recently, some researches have showed it has anti-lung cancer effect. However, the potential molecular mechanisms are unclear.

Methods: In this study, we investigated the molecular mechanisms of lappaconitine sulfate (LS) against HeLa cells.

Results: Our results suggested that LS significantly suppressed the proliferation of HeLa cells in a dose-dependent manner. Specifically, LS inhibited mitotic spindle formation through disruption of microtubule assembly and induced G1 phase cell arrest through down-regulation of the cyclin D1 level. LS also induced cell apoptosis by decreasing MDM2 expression and the ratio of Bcl-2/Bax. Additionally, LS led to constitutive downregulation of the PI3K/Akt signaling pathway and decreased the protein levels of its downstream factors, including p53, p21, p65, and caspases.

Conclusions: Collectively, the findings of our studies are that LS exhibits antiproliferation, cell cycle arrest and apoptosis-inducing effects by regulating the PI3K/Akt signaling pathway in human cervical cancer cells.

Expression of Collagen Triple Helix Repeat Containing-1 (CTHRC1) in Prostate Cancer and Correlation With Clinical Parameters

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Objectives: To investigate the expression and clinical significance of CTHRC1 gene in prostate cancer.

Methods: CTHRC1 gene expression in PCa was analyzed by gene chip, GEO database, and oncomine database. The Cancer Genome Atlas (TCGA) was analyzed by cibioportal to evaluate the prognostic role of CTHRC1 for overall survival (OS), disease-free survival (DFS). Correlations between CTHRC1 expression and clinical characteristics were assessed in 119 patient samples. To investigate CTHRC1 expression traits in PCa, we comparatively analyzed the CTHRC1 protein and mRNA profiles in PCa cell lines and and primary human normal prostate epithelial cells (HPEpiC).

Results: The expression levels of CTHRC1 were significantly up-regulated in prostate cancer tissues in two gene chip data and five oncomine datasets. In analyses of gene expression of human PCa tumor samples deposited in TCGA databases, up-regulation of CTHRC1 in human PCa patients correlated significantly with lower overall survival (OS) and disease-free survival (DFS). Immunohistochemical results showed that the positive expression rate of CTHRC1 in high-grade prostate cancer and lowgrade prostate cancer was significantly higher than that in prostat intraepithelial neoplasia (PIN) and benign prostatic hyperplasia (BPH) ($P < 0.05$). Clinical data analysis showed that there were a significant correlation between the expression intensity of CTHRC1 and the total PSA level, TNM stage, and Gleason score. ROC curve shown that as a diagnostic factor between PCa and BPH, CTHRC1 has a good positiv value ($P < 0.01$). However, as a diagnostic factor between PCa and PIN, there was no significant difference ($P > 0.05$). In addition, CTHRC1 combined with PSA can improve the ability to detect PCa ($P < 0.01$). The data showed that the CTHRC1 protein and mRNA was significantly up-regulated in PCa cell lines compared with HPEpiC, indicating that CTHRC1 may play an important role in prostate cancer development and progression.

Conclusions: The expression of CTHRC1 in prostate cancer is related to the degree of malignancy and prognosis of prostate cancer, which is expected to be a new diagnostic marker and therapeutic for prostate cancer.

Mechanistic Study of OLA1-Induced Oral Cancer Metastasis

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Objectives: Oral cancer metastasis is a life threaten problem worldwide. An effective targeted therapy is particularly important for patients with oral cancer. Our recent study suggested that OLA1 may be an effective drug target for therapy of oral cancer. This study further addressed the molecular mechanism of OLA1 in oral cancer metastasis.

Methods: Western blot was used to detect differences in OLA1 and its targeting proteins in oral cells, and qPCR was used to detect differences in RNA levels of individual proteins. Wound assay, Transwell test, MTT assay, and Flow cytometric assays were used to study the function of the OLA1 in oral cancer metastasis. Immunoprecipitation and Mass spectrometry were used to determine the mechanism by which OLA1 is involved.

Results: The endogenous level of the OLA1 in oral cancer cell lines was significantly lower than that in normal oral cells, and that in high metastatic cell line UM-1 was lower than that in the carcinoma cell line UM-2. Elevated expression of OLA1 resulted in a reduced ability of metastasis in UM-1, and the weakened resistance to paclitaxel. Knocked down OLA1 in UM-2 enhanced cell migrative ability and differentiated expression of EMT markers, suggesting that OLA1 may regulate the metastatic ability of oral cancer through the EMT pathway.

Conclusions: The above data suggest that OLA1 may be a potential target for the treatment of oral cancer and play an important role in the prognosis of oral cancer.

SIRT1/MRPS5 Axis Enhances the Metabolic Flexibility of Liver Cancer Stem Cells

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Objectives: Metabolic reprogramming endows cancer cells the ability to adjust metabolic pathway to support heterogeneously biological processes. Previous studies focus on metabolic transformation accompanied malignant degeneration of normal cells or tumor metastasis. However, the underlying regulator of the metabolic reprogramming during cancer stem cells (CSCs) differentiation remains unclear.

Methods: Here we show that liver CSCs transform mitochondrial-dependent energy supply to Warburg phenotype accompany differentiation by the dual function of mitochondrial ribosome protein S5 (MRPS5).

Results: Deacetylated MRPS5 locates in mitochondria to enhance the function of Complex-I, which increases the generation of NAD+ to enhance the oxidative phosphorylation (OXPHOS) and activate the UPRmt to treat the negative byproduct ROS, processes involved in maintenance of cancer stem cells. Conversely, acetylated MRPS5 gather in nuclei to increase the expression of glycolytic proteins and promote the Warburg Effect. The acetylation status of MRPS5 is directly regulated by deacetylatase SIRT1, which is abundant in liver CSCs and decreases during differentiation.

Conclusions: Thus SIRT1/MRPS5 axis participates in metabolic reprogramming to facilitate tumor progression and may become a promising cancer therapeutic target.

Low-Dose Microcystin-LR Antagonizes AFB1 Induced Hepatocarcinogenesis With Decrease of Precancerous Lesion and DNA Adducts Decrease

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Objectives: Aflatoxin B1 (AFB1) and microcystin-LR (MC-LR) are hepatotropic pollutants universally found especially in developing countries including China. People are exposed to those two biotoxins directly through drinking water and indirectly through food chain. Many lab experiments have proved that AFB1 and MC-LR co-exposure increase hepatocarcinogenesis. However, the combined effects of these two biotoxins on the chronic exposure at low levels remain unknown.

Methods: We established an animal model and a cell model with AFB1 and MC-LR together at different ratios, detected their carcinogenic effects in SD rats using liver histopathological analysis, examined their tumorigenicity effects in cells using CCK-8, cloning formation and animal xenograft models, and evaluated the possible mechanism by measuring liver tissues AFBO-DNA adduct formation.

Results: Our results revealed that co-exposure to AFB1 and MC-LR significantly decreased the carcinogenic effect compared with AFB1 alone as observed the SD rat liver pathological H&E staining and transmission electron microscope, and measured by the levels of serum liver function indexes (ALT, AST, GGT) and hepatocarcinoma clinical biomarkers (AFP, and area of GST-positive foci). Meanwhile, AFB1 and MC-LR in combination significantly reduced the SD rat

liver AFBO-DNA adducts expression levels of 35 weeks. Furthermore, the proliferative activity and tumorigenic ability of cells in combined exposure AFB1 with MC-LR was significantly lower than that in the AFB1 alone.
Conclusions: In conclusion, our studies show for the first time that the antagonistic effect of co-exposure to AFB1 and MC-LR on liver cancer is found in long-term chronically infected cells and animals models. And we explain that the mechanism involving in MC-LR might have reduced AFBO-DNA adducts formation. Additionally, this study provided a scientific foundation for the necessity to consider co-exposure environment pollutions when devising risk-management strategies.

Roles of the Specific circRNAs as Novel Biomarkers for Prostate Cancer

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Objectives: Objective To determine whether the differentially expressed circRNAs in prostate cancer can serve as novel biomarkers for prostate cancer diagnosis and make clear the roles of the specific circRNAs as novel biomarkers for prostate cancer during the period of early diagnosis, progress and prognosis.
Methods: A total of 173 tissue samples from the patients including 78 cases of BPH tissues and 95 cases of prostate tumor tissue were collected. We screened differentially expressed circRNAs in the SBC-ceRNA (4*180K) array using 4 pairs of paracancerous and prostate tumor tissues. The function pathways of differentially expressed circRNAs was analyzed by GO and KEGG for the host gene of circRNAs. The relative expression of circRNAs was detected by using Reverse Transcription and quantitative Real-time quantitative Polymerase Chain Reaction (RT-qPCR), and the correlation between circRNAs expression and clinicopathological features was analyzed. To predict the target miRNAs of differentially expressed circRNAs and its relevant mRNAs, we also analyzed the coexpression networks of prostate cancer according to the pattern of circRNA-miRNA-mRNA using Arraystar's homemade miRNA software, IPA, and Cytoscape 3.5.1 software.
Results: We demonstrated that 1021 differentially expressed circRNAs, of which 904 circRNAs were repressed significantly and 117 circRNAs were up-regulated. Pathway analysis showed that the host genes of differentially expressed circRNAs were mainly involved in cell adhesion, gonadotropin response, regulation of blood platelet degranulation, vitamin metabolism and amino acid metabolism. RT-qPCR analysis also confirmed the expression of hsa_circ_0062019, hsa_circ_0057558 and SLC19A1 in prostate cancer tissues were significantly up-regulated ($P < 0.01$). Further, hsa_circ_0057558 was found to be positively correlated with triglyceride level and total cholesterol, and significantly correlated with triglyceride level. The ROC curve analysis showed that the area under the ROC (AUC) of hsa_circ_0057558 and hsa_circ_0062019 were 0.729 and 0.828, respectively. The result of ROC curve analysis for the combination of PSA level and two differentially expressed circRNAs showed that AUC, sensitivity and specificity were significantly increased, which were 0.938, 84.5%, and 90.9%, respectively.
Conclusions: Our results found that differentially expressed circRNAs (hsa_circ_0062019 and hsa_circ_0057558) can be used as potential tumor markers for PCa. The depth analysis of the regulation network of the differential circRNAs in PCa will provide a direction for further understanding the mechanism and clinical diagnosis and evaluation to differential circRNAs in prostate cancer. The circRNA-miRNA-mRNA network showed that hsa_circ_0034467 and hsa_circ_0057558 regulated miR_6884; hsa_circ_0060325 and hsa_circ_0062019 regulated miR_5008. The host gene (SLC19A1) of hsa_circ_0062019 is also involved in the regulatory network, and the specific regulation mode is hsa_circ_0062019 - miR_5008-5p-SLC19A1, hsa_circ_0060325-miR_5008-5p-SLC19A1 and hsa_circ_0034467 - miR_6721-5p - SLC19A1.

Lappaconitine Sulphate (LS) Down-regulated PI3K/Akt/GSK3β Pathway and Induced Apoptosis in Human Colon Cancer Cells

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Objective: This study intended to investigate the role of lappaconitine sulfate (LS) in proliferation of human colon cancer HT-29 cells and to explore the potential molecular mechanism.
Methods: Cell proliferation was detected by CCK-8 assay and EdU proliferation assay. Cell morphological change was expressed by Hoechst 33258 staining. Expression of apoptosis related proteins were detected by Western blotting. In addition, the effect of LS on cell cycle was detected by flow cytometry.
Results: LS exhibited anti-proliferative activity and induced apoptosis in HT-29 cells in a dose-dependent manner. LS induced expression of p53, p65, and Bax, cleaved PARP, cleaved-caspase-3/7/9, and inhibited Bcl-2 expression. LS also affected cyclin D1 and p21 expression, inducing cell cycle arrest in the G0/G1 phase.
Conclusions: Our findings demonstrate that LS induced cell apoptosis, arrested cell cycle in G0/G1 phase, and suppressed the PI3K/Akt/GSK3β signaling pathway of HT-29 cells.

Artemisinin Suppresses Hepatocellular Carcinoma Growth and Migration Through Disrupting Hippo-YAP Pathway

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Objectives: Human hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. Artemisinin was clinically used as anti-malarial agents. Recently it was demonstrated to inhibit cell growth and migration in multiple cancer types. But the molecular mechanism underlying these anti-cancer activity remains largely unknown.
Methods: Western blot was used to detect protein alteration of indicated molecules. Cell proliferation assay and colony formation assay were used to test HCC cell growth in vitro. qRT-PCR analysis detected mRNA transcripts level and mtDNA copy number. Seahorse XF96 was performed to determine aerobic glycolysis and oxygen consumption rate. All the statistical data were analyzed by SPSS 16.0 (SPSS Standard version 16.0, SPSS Inc., Chicago, Ill).
Results: In this study, we focused on investigating the tumor-suppressive activity of artemisinin in HCC cells. Consistent with the previous works, we found artemisinin dramatically suppressed HCC cell growth in vitro through arresting cell cycle progression, and repressed cancer cell migration via regulating N-cadherin-Snail-E-cadherin signals. Interestingly, we found disruption of cellular bioenergetics contributes to artemisinin caused cell growth and migration inhibition. Furthermore, we also demonstrated that Hippo-YAP signal transduction was remarkably inactivated upon artemisinin administration.
Conclusions: Collectively, our data revealed a novel mechanism of artemisinin in regulating HCC cell growth and migration, which suggesting that artemisinin could be considered as a potential compound for HCC therapy.

EGF Induced TSPAN8 Up-regulating EPS8L3 Expression and Promoting Pancreatic Tumorigenesis and Progression

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Objectives: Pancreatic adenocarcinoma (PaCa) is of the major type and the fourth most common cancer-related death worldwide. TSPAN8 is one of the tetraspanins family and correlates with progression of several types of cancer representing of poor prognosis and as a tumor-associated antigen and an important angiogenesis inducer. However, the effect of TSPAN8 in PaCa remains to be clarified.
Methods: In this study, we show that higher expression of TSPAN8 in pancreatic tumor than normal tissue and correlates with poor prognosis in PaCa patients.
Results: In vitro, under EGF treatment, SOX9 can promote the TSPAN8 expression under ERK signal path. Overexpression of TSPAN8 can promote the invasion and metastasis of SW1990. On the other hand, the knockdown of TSPAN8 suppress the invasion and metastasis of Capan-2 and AsPC-1. Meanwhile, AKT is activated by EGF and phosphorylates TSPAN8 at Ser129, which in turn forms a complex with E2F7 and promotes TSPAN8 entry into nucleus for promoting EPS8L3 expression and causes the remodeling of cytoskeleton and promote cell migration. When EPS8L3 is knock down, the effect of TSPAN8

overexpression is been suppressed. In vivo, overexpression of TSPAN8 promotes tumor metastasis. If EPS8L3 is knockdown, tumor metastasis is been suppressed.
Conclusions: These findings uncover a previously uncharacterized mechanism underlying TSPAN8 enter into nucleus and EPS8L3 regulation by TSPAN8 for remodeling of cytoskeleton and promote cell migration.

GABRP Regulates Chemokine Signaling, Macrophage Recruitment, and Tumor Progression in Pancreatic Cancer Through Tuning KCNN4-Mediated Ca^{2+} Signaling in a GABA-Independent Manner

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Objectives: Neurotransmitter system has a fate-determining role for tumor progression and clinical outcome, especially for pancreatic ductal adenocarcinoma (PDAC). Here, we aimed to uncover neurotransmitters-initiated signaling pathway in PDAC cells.
Methods: The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets were used to identify differentially expressed neurotransmitter receptors. The expression pattern of Gamma-aminobutyric acid type A receptor pi subunit (GABRP) in human and mouse PDAC tissues and cells was studied by immunohistochemistry and western blotting. The *in vivo* implications of GABRP in PDAC were tested by subcutaneous xenograft model, lung metastasis model, and KrasG12D/+/ Trp53R172H/+/Pdx1-Cre (KPC) mice. Bioinformatics analysis, transwell experiment, and orthotopic xenograft model were used to identify the *in vitro* and *in vivo* effects of GABRP on macrophages in PDAC. The enzyme-linked immunosorbent assay, Co-immunoprecipitation, proximity ligation assay, electrophysiology, promoter luciferase activity, and quantitative real-time PCR analyses were used to identify molecular mechanism.
Results: GABRP expression was remarkably increased in PDAC tissues and associated with poor prognosis, contributed to tumor growth and metastasis. GABRP was correlated with macrophage infiltration in PDAC and pharmacological deletion of macrophages largely abrogated the oncogenic functions of GABRP in PDAC. Mechanistically, GABRP interacted with KCNN4 to induce Ca2+ entry, which leads to activation of NF-κB signaling and ultimately facilitates macrophages infiltration by inducing CXCL5 and CCL20 expression. Genetically inhibition of GABRP or KCNN4 suppressed macrophage infiltration and slowed growth in KPC mice.
Conclusions: Overexpressed GABRP exhibits an immunomodulatory role in PDAC in a neurotransmitter-independent manner. Targeting GABRP or its interaction partner KCNN4 may be an effective therapeutic strategy for PDAC.

A Standard Stratification Analysis in Meta-analysis: Application to Genetic Association Research on Cancer

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Objectives: Stratification analyses have been extensively used in genetic association studies to evaluate the effects of diverse factors or control for the outcomes of the confounding variables linked with a disease. There are two practical computing methods applied in previous studies, but no research paper has methodically presented computing methods and application standards for stratification analyses.
Methods: Taken referenced from the Mantel-Haenszel and Inverse-Variant approaches, we integrate those two practical computing methods and further develop them into a standard stratification method that contains two sequential steps: factorial stratification analysis and confounder-controlling stratification analysis for meta-analyses.
Results: In this paper, we first present the statistical methodology and theoretic algorithm of this standard stratification method, and then provide the application examples of genetic association meta-analyses to illustrate its computing processes and use standards. The standard stratification analysis method identifies the confounding or interacting factors, and controls for the confounding variables or calculates the strengths of the interacting effects to effectively reveal the real effects of these investigated factors on a disease in an overall study population. For instance, in the study of MDM2 polymorphism by smoking status in risk of lung cancer, we performed this standard method to help reveal the real harmful role of MDM2 polymorphism on lung cancer in the overall study population, and also to solve the abnormal phenomenon that smoking showed no association with lung cancer risk among MDM2 mutant-type carriers, which a normal meta-analysis would be failed to expose. We also discuss some other difficulties and further recommend some potential solutions concerning this method such as only partial stratified data available in practical work.
Conclusions: This standard stratification method will have broad applications in explosively grown future researches on the complicated relationships between genetics and disease or even beyond.

MicroRNA-34a Enhances Drug Sensitivity by Regulating Cell Stemness

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Objectives: Pancreatic cancer is a life-threatening disease worldwide due to high death rate. Drug resistance challenges the effective therapy of pancreatic cancer. The exact molecular mechanism underlying drug resistance still unknown.
Methods: Two cell lines including PANC-1 (less sensitive to gemcitabine) and SW1990 (most sensitive to gemcitabine) were used in this study. The sensitivity of different pancreatic cancer cell lines to gemcitabine with and without miR-34a treatment was screened by MTT assay. Stem cell surface markers CD44 and CD133 were analyzed by using qPCR and flow cytometry. Cell assays for clony formation, migration, and invasion were analyzed. The EMT pathway markers and notch1 were also studied by qPCR.
Results: The expression of miR-34a in SW1990 was significantly higher than in PANC-1. Overexpression of miR-34a in those cell lines enhanced significantly the sensitivity of pancreatic cancer cells to gemcitabine. Resuts from qPCR and flow cytometry showed that the stemness of pancreatic cancer cells was significantly reduced in cells with overexpressed miR-34a. In cell lines with the overexpressed miR-34a, gemcitabine increased inhibitory effects on pancreatic cancer cells. Overexpressed miR-34a decreased expression of the cell surface markers CD44+/CD133+, and the ability of clony formation, cell migration and invasiveness, as well as the reduced expression of the EMT markers. Target analysis further showed that notch1 is targeted by miR-34a.
Conclusions: miR-34a may affects pancreatic cancer drug-resistance by regulating cell stemness through EMT pathway.

CTAB Reversed Drug-Resistance Mediated by AMPK-HIF-1α-P-gp Pathway in Breast Cancer

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Objectives: Recently, a new concept was emerging that reprograming energy metabolism is one of hallmarks in cancer drug-resistance. CTAB (Hexadecyl trimethyl ammonium Bromide) is a known quaternary ammonium that has a potential of inhibiting mitochondrial H-ATP synthase, which is associated with energy metabolism. The effect and mechanisms of CTAB on drug-resistance are unclear. The goal of this study is to determine the possible role of CTAB in breast cancer drug-resistance associated with energy metabolism.
Methods: Cell viability of breast cancer drug-resistant cells was assessed by MTT assay. Expression of AMPK, p-AMPK, HIF-1α, and P-gp were determined by Western blot analysis. Tumor inhibition was measured by subcutaneous transplanted tumor model evaluated in nude mice.
Results: Our results showed that CTAB could overcome DOX resistance of breast cancer in vitro and in vivo. CTAB can increases DOX chemosensitivity by activation of AMPK, subsequently downregulation of HIF-1α and P-gp expression. By using compound C, an AMPK inhibitor, and HIF overexpression plasmid, it demonstrated the role of AMPK and HIF-1α playing in development of breast drug resistance.

Conclusions: Our data showed that CTAB may overcome drug resistance of breast cancer through the AMPK- HIF-1α-P-gp pathway. Our study sheds a light on development of a novel drug lead reversing drug-resistance.

CD44-α6β4 Complex Promotes Pancreatic Cancer and Metastasis

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Objectives: Pancreatic cancer is the fourth most lethal malignancy in the world. It is difficult to be detected in early phase and has remote metastatic spread. The mechanisms regulating pancreatic cancer metastasis are so far poorly understood. CD44 is a transmembrane glycoprotein expressed on a subset of pancreatic cancer cells, regulating cell migration, adhesion and many other cellular mechanisms. Integrin is a family of transmembrane receptors related to cell-extracellular matrix adhesion, intracellar skeleton and so on, which plays a role in cell signaling transduction of cell cycle. α6β4, as a subunit of integrin, was found a receptor of CD44. Thereby, our study was to answer the question whether the CD44-α6β4 complex may lead the activation of EMT associated downstream genes so as to promote the pancreatic cancer development and metastasis.

Methods: Based on the data in TCGA database, the CD44 expression level between normal population and pancreatic carcinoma patients and the survival curve of patients with high or low/medium CD44 expression levels were explored, obviously showing that patients with high CD44 expression levels had poor prognosis.

Results: The expression of CD44 in pancreatic cancer was positively correlated with α6β4 according to the result of western blot of blood serum samples. Outcomes from databases and clinical samples suggest that CD44 might promote tumor metastasis via contacting with a6b4. These findings showed that CD44 was found to be an independent predictor of prognosis in tumor and survival analysis. EMT is an essential cellular process in carcinoma pathogenesis. It plays an important role in forming highly malignant tumors which have evident motility, invasiveness and higher chance of developing distant metastases. We found that CD44 could regulate the expression of EMT associated proteins. α6β4 could be evidently seen downregulated with the CD44 knockdown according to the western blot and co-localization assay. Abnormal cell proliferation is an essential point in tumor growth and aggressiveness. It is supposed that the EMT-related protein, CD44, could also affect tumor cell proliferation. Clonogenic assay, proliferation assay and apoptosis assay were conducted to prove that CD44 affects cell proliferation and viability. Exosomes were known as essential messengers promoting cancer metastasis, thus we hypothesized CD44 in exosomes could also play a role in remote metastasis process. To explore whether the CD44-α6β4 complex also plays a role in exosome delivery, CO-IP was conducted to prove the binding of CD44 and α6β4.

Conclusions: Migration assay of target cells with the treatment of co-culture of wildtype and CD44kd exosomes displayed that CD44 could promote the cancer cell invasion. Taken together, CD44 and α6β4 formed a complex in an intercellular or exosome-delivered way to promote pancreatic cancer development and metastasis.

To Analyze the Result of Nuclear Matrix Protein 22 Assay in Urine From Zhoupu Region in Shanghai

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Objectives: A retrospective review the situation and related conditions of the patients of detection result of nuclear matrix protein 22 (NMP22) and routine urine parameters, detection of blood parameters, provide clues and evidences for diagnosis of bladder cancer.

Methods: The medical record data of patients who were detected on NMP22 were collected from July 2014 to July 2018 in Shanghai University of Medicine & Health Sciences Affiliated Zhoupu Hospital, and were carried out by statistical analysis.

Results: The positive rate of NMP22 assay was 23.88%, the mean age of NMP22-positive patients was (61.35 ± 18.73) years, while the mean age of NMP22-negative was (58.92 ± 17.43) years, Logistic regression analysis showed that there were significant correlations between NMP22-positive and the ages of patients, strong positive of proteinuria, urinary erythrocyte and urinary leukocyte in routine urine, exceed cut-off value of C reactive protein (CRP) and serum amyloid A (SAA). What the corresponding OR value were 1.141, 1.344, 1.249, 1.320, 1.716, 1.857, and corresponding *P* value were 0.023, 0.006, 0.040, 0.011, 0.009, 0.003, respectively.

Conclusions: The NMP22-positive may be correlated with many factors such as the ages of patients, strong positive of proteinuria, urinary erythrocyte and urinary leukocyte in routine urine, exceed cut-off value of CRP and SAA; NMP22 assay combined with routine urine parameters and blood parameters had important significance in screening examination of bladder cancer.

S1P/S1PR3 Controls Osteosarcoma Growth Through YAP-Dependent Aerobic Glycolysis

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Objectives: The Hippo pathway plays essential roles in organ size regulation and cancer prevention via restricting its downstream transcriptional co-activator, Yes associated protein (YAP). Previous studies have revealed an oncogenic function of YAP in reprogramming glucose metabolism, which is a hallmark of cancer cells. However, the underlying mechanism remains largely unknown.

Methods: Here, we provide evidence that S1P receptor 3 (S1PR3) plays an important role in the regulation of YAP-dependent glycolysis during osteosarcoma (OS) growth.

Results: S1P and S1PR3 are upregulated in OS, and higher expression of S1PR3 is associated with worse survival rate. Furthermore, in vitro and in vivo experiments demonstrate that the S1P/S1PR3 axis promotes proliferation, inhibits apoptosis, and contributes to the Warburg effect in OS cells. Mechanistically, the S1P/S1PR3 axis inhibits the phosphorylation of YAP and promoting nuclear translocation of YAP, which contributes to the formation of YAP-c-MYC complex and enhances the transcription of glycolysis activator PGAM1. Taken together, our study reveals a previously unappreciated function of S1P/S1PR3 signaling in OS, which is intertwined with YAP signaling and metabolic alterations.

Conclusions: Targeting the S1P/S1PR3 axis may be a potential metabolism-targeting therapeutic approach for patients with OS.

TSPAN8 Promotes Cancer Cell Stemness via Activation of Sonic Hedgehog Signaling

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Objectives: Cancer stem cells (CSCs) represent a major source of treatment resistance and tumor progression. However, regulation of CSCs stemness is not entirely understood. Tetraspanins are a family of 33 members in mammals, including TSPAN8 (encoded by TSPAN8 gene) and several clusters of differentiation (CD) proteins, which play major roles in a plethora of cellular functions. Increasing evidences suggest that TSPAN8 promotes tumor cell migration, invasion and metastasis in different types of human cancers.

Methods: Here, we demonstrate that TSPAN8 expression is upregulated in breast CSCs, promotes the expression of the stemness genes NANOG, OCT4, and ALDHA1, and correlates with therapeutic resistance.

Results: Mechanistically, TSPN8 interacts with PTCH1 and inhibits the degradation of the SHH/PTCH1 complex through recruitment of deubiquitinating enzyme ATXN3, resulting in the binding of protein kinase GRK2 to SMO, phosphorylation and translocation of SMO to cilia, GLI1 activation for downstream gene expression, cancer cell stemness, resistance of CSCs to chemotherapeutic agents, and enhances tumor formation in mice. Accordingly, TSPAN8, PTCH1, SHH, and ATXN3 are relatedly expressed in human breast cancer specimens, and TSPAN8 and ATXN3 expression correlates with poor prognosis.

Conclusions: These findings reveal a molecular basis of TSPAN8-enhanced Sonic Hedgehog signaling and highlight an important role of TSPAN8 in promoting cancer cell stemness.

Anticancer Compounds From Starfish Regenerating Tissues and Their Antioxidant Properties on Human Oral Epidermoid Carcinoma KB Cells

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Objectives: Starfish extract was previously evaluated by our research group for its wound restoration performance. Due to its complex structure of star fish tissue regeneration capacities, attempts have been made to analyze its bioactive components from amputated tissue extracts for anticancer and antioxidant efficacy. Our prior studies have shown the significant effects of starfish extracts in regenerating damaged tissue in zebra fish. However, the active fractions and unique cellular mechanisms of starfish regenerated tissues extracts have yet to be elucidated. The goal of this study was to examine the anti-proliferative and antioxidant effects of bioactive compounds from *L. maculata* and dissect its mechanism of action.
Methods: Cytotoxicity on human carcinoma KB cells was measured by MTT assay. To gain more insight of the mode of anti-proliferative impact of starfish extracts, we quantified intracellular ROS levels by DCFH-DA, mitochondria membrane ability alterations by Rh-123 staining, oxidative DNA damage through comet assay and apoptotic morphological changes through AO/EtBr dual staining technique. The pharmacologically active components have been characterized by HPLC, GC-MS/MS, and FTIR.
Results: Our key findings showed that the bioactive fraction acquired from HPLC induced apoptosis, enhanced ROS levels, altered the mitochondria membrane potential and increased the oxidative DNA damage in KB cells. The up-regulation of Bax/Caspase 3 protein expression was negatively correlated with the expression of Bcl-2 protein and the proliferation of cyclin D1 associated markers in active components treated KB cells. Furthermore, 35 fractions with predominant anticancer compounds have been determined as anticancer compounds by GC-MS, including 5α-Cholest-7-en-3β-ol, Hexadecanoic acid, Myo-Inositol, 9,12-Octadecadienoic acid, and 9,12,15-Octadecatrienoic acid, which could be responsible for the anti-proliferative impact of KB cells.
Conclusions: Our data suggest that bioactive compounds from regenerated tissues of *L. maculata* starfish extracts exhibits potent anticancer effect in KB cells, which could be promising assets of anticancer, antioxidant and wound healing agents in biomedicine.

Detection of HER2 Amplification Using Multiple Reference Genes by 3-color Digital PCR

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Approximately 20% of current HER2 testing may be inaccurate, partly because of polysomy 17 and centromeric amplification. Here three reference genes were assessed using 3-color digital PCR to evaluate HER2 amplification in FFPE samples. The concordance rate of HER2 amplification examined in FFPE samples with HER2/CEP17 dPCR assay and IHC/FISH was 84% (3 out of 19), and those with HER2/3-ref and IHC/FISH was 94.7% (1 out of 19). It demonstrated that this dPCR method was as effective as IHC/FISH and therefore might present an effective way to determine the true HER2 amplification status for guiding HER2-targeted therapy.

A Next Generation Sequencing Panel (CNSeq) for Molecular Classification of Central Nervous System Tumors

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Objectives: The molecular classification of CNS tumors is important for clinical management.
Methods: A targeted panel (CNSeq) covering 348 amplicons for SNVs, MGMT methylation and 1p19q LOH derived from 13 genes frequently aberrant in different glioma types were designed.
Results: Performance of CNSeq assay were evaluated in 43 CNS tumors, and CNSeq assay correctly identified 43/43 (100%) SNVs and methylation variation and 42/43 (97.7%) 1p19q LOH known to be present by conventional techniques, including *IDH1,IDH2,BRAF,TERT,1p19q LOH* and *MGMT* methylation.
Conclusions: It allows rapid and cost-effective profiling of brain tumor specimens and thus provides valuable information for patient management.

Up-regulation of OLA1 Promotes the Stemness and EMT Phenotypes in Chemoresistant Pancreatic Cancer Cells

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Objectives: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignant disease with a 5-year survival rate less than 5% mostly because of the drug resistance. Cancer stem cells (CSCs) and epithelial–mesenchymal transition (EMT)-type cells are considered as potential causes of chemoresistance, tumor recurrence and metastasis in pancreatic cancer. Obg like ATPase 1 (OLA1) is a newly cloned member of the Obg family P-loop GTPases, which is overexpression in many malignant tumors. In this study the potential role of OLA1 in the acquired drug resistance of pancreatic tumor was investigated.
Methods: Methyl thiazolyl tetrazolium (MTT) assay, qRT-PCR and Western blot (WB) analysis were used to verify the relationship between OLA1 and chemoresistance. Flow cytometric assay (FCA), tumor sphere formation, clony formation, qRT-PCR and WB assay were used to detect the stemness and Epithelial-mesenchymal transition (EMT) marker in the OLA1 regulated cell lines.
Results: In the present study, we focused on investigating how OLA1 involved in the chemoresistance of pancreatic cancer. We found that OLA1 overexpression in the chemorisistant patients indicated OLA1 induced chemoresitance through EMT pathway. Down-regulated OLA1 improved the sensitivity of Gemcitabine and reduced stem cell markers (CD44+, CD133+, c-Met), and EMT (snail + slug, E-cadherin) process in pancreatic cancer cells. These phenomena indicate that OLA1 confers the chemoresistance of PDAC and may serve as a potential target for therapy of pancreatic cancer.
Conclusions: Results observed-above suggest that OLA1 was associated with Gem resistance of pancreatic cancer. Exact molecular mechanism underlying OLA1 regulation in pancreatic cancer resistance is still under investigation.

Mitochondrial Matrix Peptidase ClpP Regulates ROS-mediated MAPK Activation to Promote Esophageal Squamous Carcinoma Tumorigenesis

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Objectives: Esophageal squamous carcinoma (ESCC) is one of the most aggressive malignancy in digestive system with high rate of mortality worldwide. ClpP as an important mitochondrial protease, the inhibition of ClpP can impair tumor growth and drug resistance in some cancer types. However, the role of ClpP in ESCC development remains largely unknown.
Methods: Western blot and qRT-PCR were used to detect protein and mRNA alteration of indicated molecules. Seahorse XF96 was performed to determine aerobic glycolysis and oxygen consumption rate. IHCwas used to analyze ClpP protein level in ESCC tumor tissues and adjacent non-cancerous tissues. Kaplan-Meier survival analysis was applied to analyze survival rate post-operation.
Results: In the present study, we focused on investigating how ClpP involved in the development of ESCC. We found that ClpP overexpress in most ESCC patients, which play a protective role in tumor progression. Down-regulated ClpP can inhibit cell growth and migration. Additionally, we found that ClpP down-regulated in ESCC cells decreased cellular bioenergetics as determined by measuring aerobic respiration and glycolysis using extracellular flux analyzer. Furthermore, depletion of ClpP can suppress autophagy and MAPK pathway by down-regulating ROS production. We also demonstrated that ClpP was an independent prognostic factor for overall survival of ESCC.
Conclusions: Taken together, these findings indicate that ClpP contributes to the development of ESCC and may serve as a potential biomarker for diagnosis and prognosis of ESCC and therapeutic target for patients with ESCC.

In Vitro Metabolic Diagnosis of Early Stage Lung Cancer Using Plasmonic Gold Chips

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Objectives: In vitro diagnostics (IVD) contributes to ~66% of clinical diagnosis serving as the "eye" of medical doctors. IVD analysis relies on rationally designed materials for direct metabolite detection with minimal sample treatment procedures for practical diagnostic applications. However, current metabolic analysis face major challenges including (a) the low molecular abundance and high sample

complexity of metabolites in biofluids; and (b) construction of diagnostic tools for real case application in clinics.

Methods: We constructed a series of chips coated with plasmonic gold nanoshells for metabolic fingerprinting of biofluids and exosomes in lung cancer diagnostics by laser desorption ionization mass spectrometry (LDI MS). The surface roughness of chips was achieved through controlled particle synthesis, dip-coating, and gold sputtering for mass production. We integrated the optimized chip with microarrays for laboratory automation and micro-/nanoscaled experiments, affording sensitive, selective, multiplex, and quantitative metabolic fingerprinting using 500 nL of serum, cerebrospinal fluid (CSF), urine and exosomes. We further utilized these optimized plasmonic chips for in vitro metabolic diagnosis of early stage lung cancer patients using serum and exosomes.

Results: We constructed the plasmonic chip through a three step process including particle synthesis, dip-coating, and gold sputtering. The as-made chip demonstrated reproducible and high-throughput metabolic analysis using biosamples ranging from 400 nL down to 400 pL. The distinct surface roughness on-chip present due to the specific nanogaps and nanocrevices of gold shells selectively trapped small metabolite molecules and transfer the laser energy, toward advanced metabolic analysis of complex biosamples in real case. The on-chip metabolic fingerprinting of serum/CSF/urine and exosomes, by direct LDI MS demonstrated the selectivity toward small metabolites in a complex bio-mixtures compared to conventional methods. We further differentiated the early stage non-small cell lung cancer (NSCLC) patients from healthy controls by on-chip metabolic analysis of serum and exosomes, and anticipated these key m/z values to serve as potential metabolic markers.

Conclusions: We have introduced plasmonic gold chips as new substrates for direct LDI MS detection of small metabolites in biofluids and exosomes, and further constructed a novel platform technology for metabolic fingerprinting based IVD. Our work makes solid contributions to the design of materials (gold nanoshells) and device (plasmonic chips) for advanced metabolic analysis toward precision medicine, and initiates the development of various personalized diagnostic tools for diverse diseases including but not limited to lung cancer in the near future.